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MICROORGANISMS AND ENVIRONMENT MANAGEMENT

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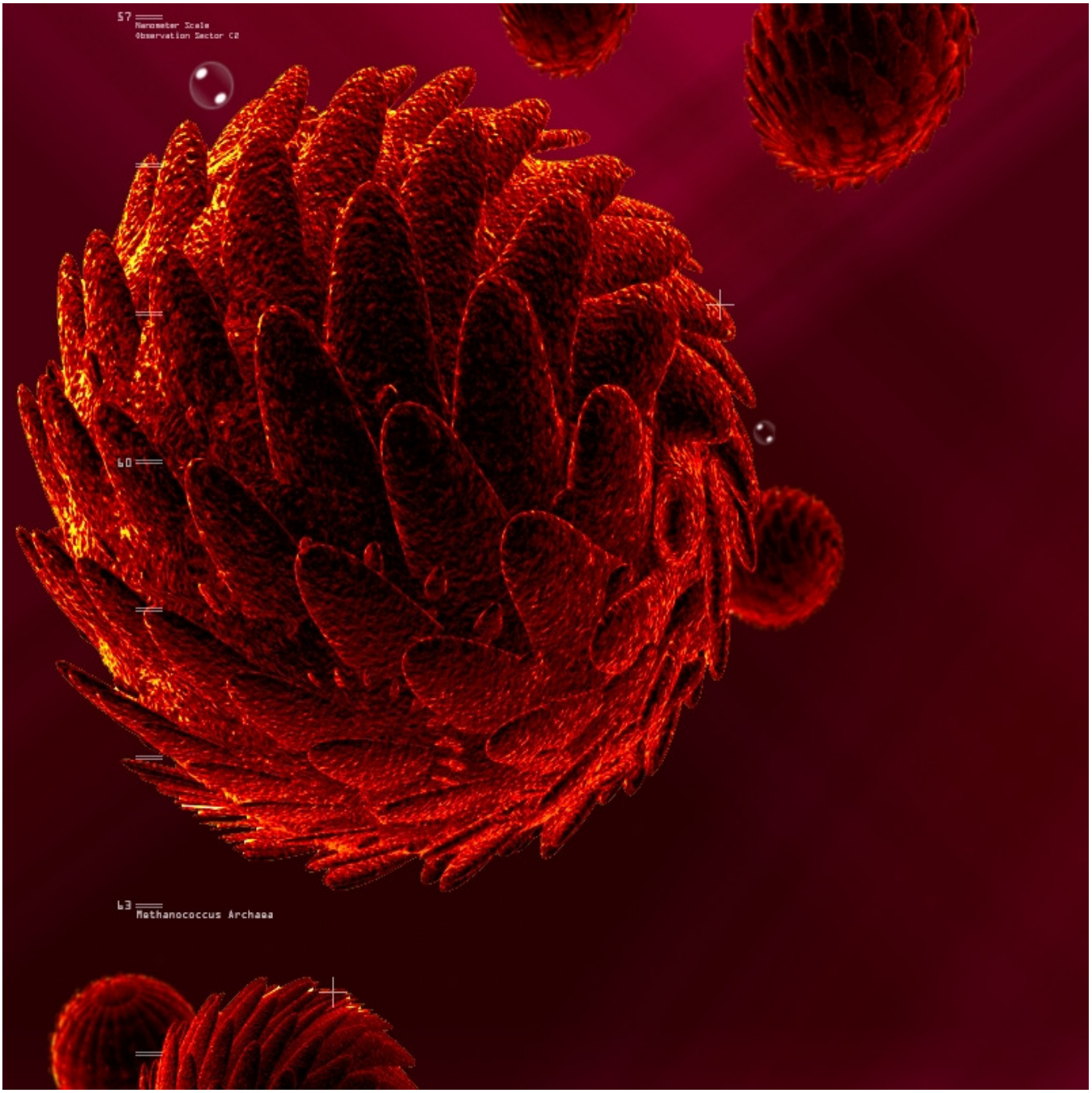

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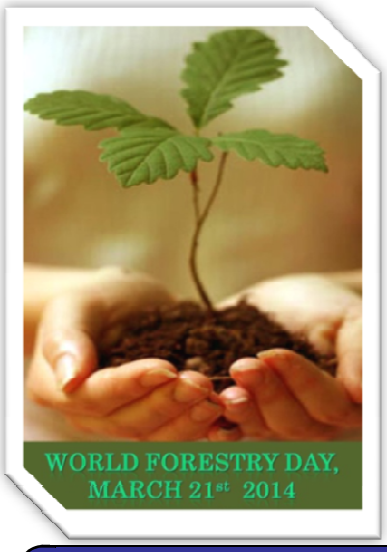

INSTRUCTIONS TO CONTRIBUTORS

ENVIS Newsletter on 'Microorganisms and Environment Management', a quarterly publication, brings out original research articles, reviews, reports, research highlights, news-scan etc., related to the thematic area of the ENVIS Centre. In order to disseminate the cutting-edge research findings to user community, ENVIS Centre on Microorganisms and Environment Management invites original research and review articles, notes, research and meeting reports. Details of forthcoming conferences / seminars / symposia / trainings / workshops also will be considered for publication in the newsletter.

The articles and other information should be typed in double space with maximum of 8 - 10 typed pages. Photographs/line drawings and graphs need to be of good quality with clarity for reproduction in the newsletter. For references and other details, the standard format used in refereed journals may be followed.

Articles should be sent to:

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Cover page : *Methanococcus jannaschii,* an autotropic hyperthermophillic Archaea, belongs to a specific group called methanogens. *M. jannaschii* became the first organism in the archaea to have its complete genome sequenced.

ENVIS Newsletter
on
Microorganisms and Environment Management

Contents

Dear Readers,

New Year Greetings - 2014!

Antibiotics, also known as antibacterials, are types of medications that destroy or slow down the growth of bacteria. Antibiotics are used to treat infections/illness caused by bacteria. The first antibiotic, penicillin, was discovered in 1929 by Sir Alexander Fleming. There exists several penicillin-related antibiotics as ampicillin, amoxicillin and benzylpenicilllin and are widely used to treat a variety of infections.

In the past 6 decades or so, they have played a crucial role in fighting against infectious diseases caused by bacteria and other microbes. Antimicrobial chemotherapy has been a leading cause for the increase of average human life span in the 20th Century. However, certain pathogenic microbes (that cause wound infections, gonorrhea, tuberculosis, pneumonia, etc.) have developed resistant, at genetic level, against antibiotic drug therapy. Several reasons could be attributed for such resistance, that they are remarkably resilient and development of resist antibiotics and other antimicrobial drugs. The increased use and misuse of existing antibiotics in human and veterinary medicine and in agriculture are the prime factor for their resistance. Microbial development of resistance, as well as economic incentives, have resulted in research and development in search for new antibiotics in order to maintain a pool of effective drugs at all times.

In this context, this issue highlights evolution of environmental microbial population on antibiotic resistance, its secretary system and drug resistance mechanisms. Other interesting reports on antibiotic resistance are also included.

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Prof. N. Munuswamy

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SCIENTIFIC ARTICLE

Evolution of environmental microbial population in the emergence and global spread of antibiotic resistance

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Abstract

Antibiotic resistance has become a major public health issue for today's human being. Globally, antibiotic resistance affects more people than the deadly diseases like HIV-AIDS. Environmental microbiota play a major role in the origin and spread of antibiotic resistance. The role of environmental microbiota in antibiotic resistance is not well acknowledged. Therefore, this paper documents the role of environmental microbes in the emergence and global spread of antibiotic resistance.

Introduction

Bacteria were the first form of life to appear on earth, about 3.5 billion years ago and as a consequence they have acquired and learned the essentials of survival. From a Darwinian perspective, this ability to survive forms the basis for a successful evolution. This in turn facilitates their survival in the harshest of environments on earth.

Antibacterial therapy has emerged over the last 70 years and become the mainstay of treatment in modern medicine. Despite this, we have seen the development of antibiotic resistance even in the early 20th century when penicillin was discovered by Alexander Fleming. The refinements of antibiotic resistance is yet to be fully deciphered, one of the main reasons for this is the high level of complexity involved with antibiotic resistance both from a genetic and ecological perspective (Sykes, 2009).

The evolution of antibiotic resistance provides an ideal example of the latin term "*ex unibus plurum* (towards diversification) and *ex pluribus unum* (towards unification)" as many of the evolutionary units of antibiotic resistance have oscillatory dynamics. This further complicates our understanding of antibiotic resistance (Baquero, 2011).

Thus, antibiotic resistance is not only the consequence of genetic variation, but also a cause of such genetic variation. What we detect as molecular observers of antibiotic resistance are correlated changes in the frequency in entities as resistance genes, other genes, plasmids, clones, species or bacterial communities (Sykes, 2009).

In recent years, antibiotic resistance genes and the carriers of the resistant genes from non-clinical environment have gained more attention. It is largely due to the "butterfly effect" of rare genetic events, that are hypothesised to have occurred in the non clinical environment.

It is further substantiated by a current school of thought which states that antimicrobial-resistance genes and their genetic vectors, once evolved in bacteria of any kind, anywhere, can spread indirectly through the world's interconnecting commensal, environmental, and pathogenic bacterial populations to other kinds of bacteria elsewhere.

Survival of the best connected

An emerging concept increasingly used in Public Health Microbiology is 'high risk clones or clonal complexes', referring to highly specialised genetic populations with enhanced ability to colonize, spread and persist in particular niches after acquiring adaptive characters including antibiotic resistance and replacing the antibiotic susceptible population. High connectivity promotes rapid Horizontal Gene Transfer (HGT). If a bacterium belongs to a preferential genetic exchange community, the probability of further evolution by HGT increases. This holds true, particularly for antibiotic resistant traits as it leads to rapid dissemination of genes (Baquero and Coque, 2011).

Genetic carriers of resistant genes

The carriers of antibiotic resistance consist of a complex system of genetic entities which are embedded within genetic elements of larger scale complexity. The overall picture is more complicated not only because of the number of elements involved, but also due to the heterogeneity of such components and their hierarchical organization.

Resistance genes are most often encoded in extrachromosomal genetic elements or in segments that appear to have been recombined into the chromosome from other genomes. The largest of the extra-chromosomal elements are the plasmids, which are self-replicating, double-stranded helices of DNA, some of which are involved in the transfer of the plasmid to another bacterial cell. Bacteria isolated from patients, about 70 years ago or more, before antimicrobials were first used, had plasmids similar to those seen now, but,

then, the plasmids had no resistance genes. This indicates that plasmids are a subunit of bacterial evolution. Further, recent studies analyzing a few sequenced plasmids indicates that they contain an assortment of genetic elements with G+C content indicative of a wide array of living organisms including bacteriophages and environmental bacteria (Bonnet, 2004).

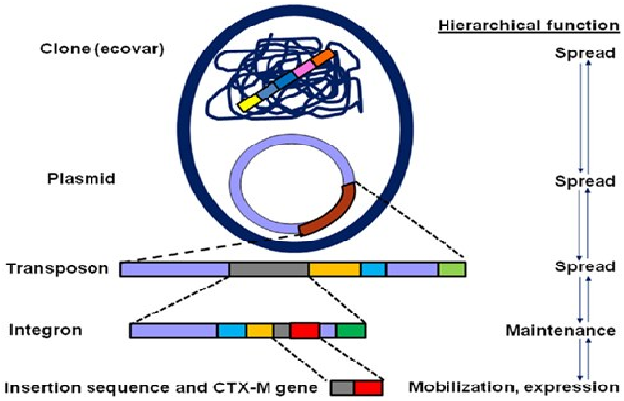
Resistance genes encoded in plasmids are often located within the segments, called transposons. Functioning transposons, include transposases, that enable the transposon to recombine into other genomes. Resistance genes are often further clustered within the elements called integrons, which are frequently found within transposons and plasmids, but also found in bacterial chromosomes. Each resistance gene in an integron is encoded in a mobile gene cassette that can be excised and then incorporated into another integron on another genome (O'Brien, 2002).

Antibiotic resistance and environmental bacteria

Although use of antibiotics for treating bacterial infection was a human intervention, antibiotics were an adaptation by the environmental bacteria to give them a competitive edge. In the game of one-upmanship, to occupy a particular ecological niche, environmental bacteria produce the antibiotic and the resistance mechanism to counter it. The wide array of above-mentioned genetic carriers mobilise the genes responsible for antibiotic resistance into the clinical environment. This is analogous to using civilian nuclear supplies for military operations. This is one facet of the overall scheme (Toleman and Walsh, 2011).

Environmental bacteria as a reservoir of clinically relevant resistant genes

Environmental bacteria are proven potential reservoir of clinically resistant genes, however direct evidence has been provided only for a few resistant genes in gram-negative bacteria. Important and significant among the environmental bacteria are the *Kluyvera spp.,* a soil environmental bacteria of the family *Enterobacteriaceae,* which is considered as a reservoir of the *CTX M* gene family that encodes resistance to 3rd generation cephalosporins and *Shewenella algae,* a marine bacterium, is the reservoir of a quinolone resistance gene, *qnr A*. The mobilization of the genes from environmental bacteria to clinical pathogens is incremental in nature. It was catalysed by a few one off events (Toleman and Walsh, 2011).

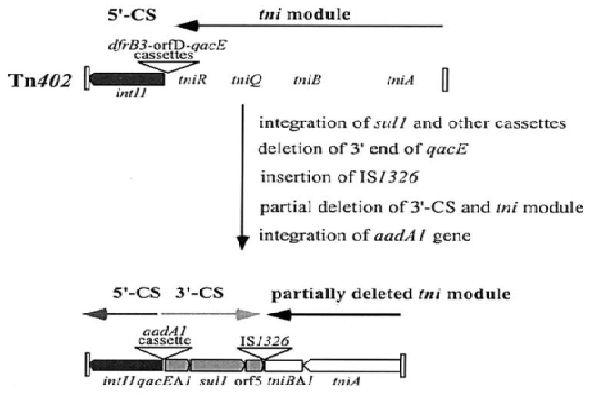


Hierarchical organization of various genetic elements

Rare genetic one-off events in gram negative bacteria

There are three key one-off events that are at the heart event of current HGT of resistance genes in Gram-negative bacteria and ultimately necessary for the construction of large extended resistance islands such as (1) capture of the class 1 integrase gene and the attI1 site by the ancestor of the Tn5090/Tn402 transposon; (2) the formation of the 3' conserved segment (3'-CS) by the fusion of the disinfectant resistance gene cassette *qacE* to a gene conferring sulphonamide resistance *sul1* forming the fused genes *qacEΔsul1* and (3) fusion of ISCR1 to the class 1 integron via *qacEΔsul1* (Toleman and Walsh,2011).

All these events represent genetic recombination that occurs randomly. Only those that confer a selective advantage are maintained successfully which gives scope for further evolution. Though it is not possible to determine the exact time and place of the genetic recombination, it is possible to hypothecate only based on continuous observations. For instance, the transposon Tn 402 is a mercury resistant transposon which was used as antiseptic in the late 19th century. Further the *qac E* gene codes for quaternary ammonium compound resistance which was used as antiseptic in the early 20th century. The *sul 1* gene fusion is believed to have occurred in the beginning of the antibiotic era because of the selective advantage that is conferred by deactivating sulphonamides, which were the first antimicrobials used. Further, the recently identified gene cassettes encode resistance to newer classes of antibiotics which indicates the emergence of class 1 integrons.



Structure of Class 1 Integron

Integrons and Environmental bacteria

Among the different mechanisms involved in lateral genetic transfer, the class 1 integrons are one of the most successful elements in the acquisition, abundance, maintenance and spread of antimicrobial resistance gene cassettes among gram-negative bacilli. Class 1 integrons are considered to be a molecular fossil to study the chronological evolutionary events in the development of antibiotic resistance because of their basic structure (Nardelli *et al.,* 2012).

Overall, it is assumed that 2.65% of eubacterial cells in non-clinical samples contain a class 1 integron. However, factors involved in the distribution of non-clinical class 1 integrons within natural communities remain largely unknown. What is known with certainty is that the class 1 integrons confer a benefit to the host cell due to their ability to acquire gene cassettes that could provide advantages for survival in hostile environments. Hence, integrons are considered to be a surrogate marker of multiple antibiotic resistant traits which get disseminated via HGT.

A recent pilot study conducted on the drinking and seepage water samples in and around Chennai (unpublished data), indicated a low prevalence of class 1 integrons among cultivable environmental bacteria. Further, 6/7 class 1 integrons possessed novel 3‘ conserved segment indicative of further genetic recombination events in the non-clinical environment.

Since cultivable bacteria form a minor part of the non clinical environmental samples including water samples, metagenomic approach might reveal a true picture about antibiotic resistance and the non clinical environment.

Conclusion

Research focusing on the role of non-clinical environment needs to be carried out to know the role of these environments in the emergence and spread of antibiotic resistance which would give an understanding and pave the path for future therapeutic interventions based on ecology and evolution.

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RESEARCH REPORTS

Spread of antibiotic resistance understood by unraveling bacterial secretion system

between bacteria and therefore the spread of antibiotic resistance has been uncovered by a team of scientists at Birkbeck, University of London and UCL.

The study, published in *Nature*, reveals the mechanism of bacterial type IV secretion, which bacteria use to move substances across their cell wall. As type IV secretion can distribute genetic material between bacteria, notably antibiotic resistance genes, the mechanism is directly responsible for the spread of antibiotic resistance in hospital settings. It also plays a crucial role in secreting toxins in infections causing ulcers,

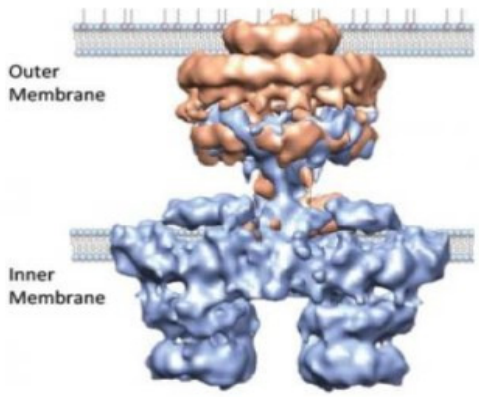
whooping cough, or severe forms of pneumonia such as Legionnaires' disease.

The work, led by Professor Waksman at the Institute of Structural and Molecular Biology (a joint Birkbeck/UCL Institute) and funded by the Wellcome Trust, revealed that the type IV secretion system differs substantially from other bacterial secretion systems, in both its molecular structure and the mechanism for secretion.

Professor Waksman said “This work is a veritable tour de force. The entire complex is absolutely huge and its structure is unprecedented. It is the type of work which is ground-breaking and will provide an entirely new direction to the field. Next, we need to understand how bacteria use this structure to get a movie of how antibiotics resistance genes are moved around.”

Using electron microscopy the team was able to reconstruct the system as observed in the bacteria *E. coli*. They saw that the mechanism consists of two separate complexes, one in the outer membrane of the cell, and the other in the inner membrane, which are connected by a stalk-like structure that crosses the periplasm, the space between the two membranes. The complexes both at the inner and outer membranes form pores in the membrane, via which substances can be secreted.

Understanding the structure of the secretion system will help scientists to uncover the mechanism by which it moves substances across the inner and outer membranes. It could eventually help scientists develop new tools for the genetic modification of human cells, as the bacteria could act as a carrier for genetic material, which could then be secreted into cells.



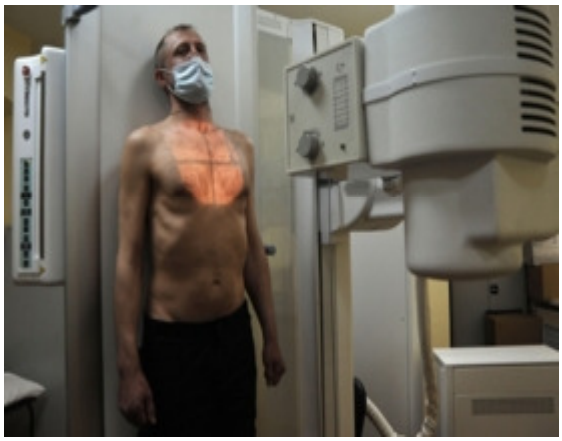
Bacterial type IV secretion system structure reveals how antibiotics resistance genes move from one bacterium to another.
(Image Credit: Image from the Nature paper)

Professor Waksman said: "Understanding bacteria's secretion system could help design new compounds able to stop the secretion process, thereby stopping the spread of antibiotics resistance genes. Given that antibiotics resistance has become so widespread and represents a grave threat to human health, the work could have a considerable impact for future research in the field of antimicrobials.”

Source: www.sciencedaily.com

Drug-Resistant Tuberculosis from Russia Is Spreading More Easily

Bacterial 'superbugs' are getting ever more potent. Tuberculosis (TB) strains in Russia carry mutations that not only make them resistant to antibiotics but also help them to spread more effectively, according to an analysis of 1,000 genomes from different TB isolates — one of the largest whole-genome study of a single bacterial species so far.



Newly discovered mutations are helping tuberculosis to stay infectious while evolving resistance to multiple drugs.

TB, which is caused by the bacterium *Mycobacterium tuberculosis*, exploded in Russia and other former Soviet nations in the early 1990s, after the collapse of the Soviet Union and its health system. The incomplete antibiotic regimens some patients received, meanwhile, sparked rampant drug resistance. But the latest study of TB cases in Russia, published in *Nature Genetics*, indicates that such ‘programmatic’ failures may not be the only explanation for the rise of drug-resistant TB in the region — biological factors also play a big part.

As part of a long-standing effort to study the rampant drug-resistant TB in Samara, a region of Russia about 1,000 kilometers southeast of Moscow, researchers collected TB isolates from 2,348 patients and sequenced the entire genomes of 1,000 of them. This enabled the team to identify previously

unknown mutations linked to antibiotic resistance, as well as 'compensatory mutations' that improve the ability of drug-resistant TB to spread.

Nearly half of the TB isolates were multi-drug resistant, which means that they were impervious to the two common first-line antibiotics that cure most TB infections, while 16% of these isolates also harbored mutations that made them impervious to 'second-line' drugs. These infections are more expensive to treat, and patients who receive ineffective drugs are more likely to spread TB.

"It certainly adds an extra layer of worry, because one had assumed if you could solve programmatic weaknesses, you would solve the problem of the drug-resistant TB," says the study's lead author Francis Drobniewski, a microbiologist at Queen Mary University of London. "But this does seem to be a biological problem as well."

"Although we know the general story of TB drug resistance in Russia, these new findings are still shocking," says Christopher Dye, an epidemiologist at the World Health Organization in Geneva, Switzerland. "Truly scary," he adds.

Antibiotics block essential functions in bacteria, such as making proteins or building cell walls. Mutations in the genes involved in these duties can lead to antibiotic resistance, but they also tend to make bacteria divide more slowly. But laboratory experiments have shown that bacteria can develop compensatory mutations that restore the pathogen's ability to divide quickly. Drobniewski's team found such mutations in more than 400 isolates that were resistant to the first-line antibiotic rifampicin, and the authors suggest that the mutations might overcome the growth-slowing effect of evolving resistance.

"The worst scenario is that the organisms are developing resistance, compensating for it, and evolving into something that's new and different, that's much less treatable," says Megan Murray, an epidemiologist at the Harvard School of Public Health in Boston, Massachusetts. In an earlier study, her team found both widespread drug resistance and compensatory mutations in their analysis of 123 TB genomes from around the world.

But even if biology is a major driver of Russia's drug-resistant TB epidemic, public-health officials can still beat it back, says Dye. "My bet is that, if the local control programme correctly identifies strains carried by each patient, and treats them with the most effective drug regimens, then the number of resistant cases will fall," he says. "That's what we've seen in Estonia, Hong Kong, the USA and elsewhere. I doubt that Russia is different."

Source: www.scientificamerican.com

ONLINE REPORTS ON MICROORGANISMS

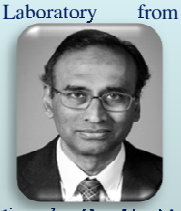
New functions for 'junk' DNA?

DNA is the molecule that encodes the genetic instructions enabling a cell to produce the thousands of proteins it typically needs. The linear sequence of the A, T, C, and G bases in what is called coding DNA determines the particular protein that a short segment of DNA, known as a gene, will encode. But in many organisms, there is much more DNA in a cell than is needed to code for all the necessary proteins. This non-coding DNA was often referred to as "junk" DNA, because it seemed unnecessary. But in retrospect, we did not yet understand the function of these seemingly unnecessary DNA sequences.

We now know that non-coding DNA can have important functions other than encoding proteins. Many non-coding sequences produce RNA molecules that regulate gene expression by turning them on and off. Others contain enhancer or inhibitory elements. Recent work by the international ENCODE (Encyclopedia of DNA Elements) Project suggested that a large percentage of non-coding DNA, which makes up an estimated 95% of the human genome, has a function in gene regulation. Thus, it is premature to say that "junk" DNA does not have a function -- we just need to find out what it is!

KNOW A SCIENTIST

Dr. Venkataraman Ramakrishnan, an Indian-born American & British citizen, carried out his post doctoral research on ribosomes after obtaining Ph.D. degree in Physics, as a staff scientist at Brookhaven National Laboratory from 1983-95. Ramakrishnan and colleagues published a 5.5 Angstrom resolution and complete molecular structure of the 30S subunit of ribosome and its complexes with several antibiotics and determined the atomic structure of the whole ribosome in complex with its tRNA and mRNA ligands. **Dr. Venki Ramakrishnan** is also known for his past work on histone and chromatin structure.



He was honored with **Nobel Prize in Chemistry in the year 2009** for his contribution on **structure and functions on the ribosome**, along with **Thomas A. Steitz** and **Ada E. Yonath**, at the MRC Laboratory of Molecular Biology in Cambridge, England.

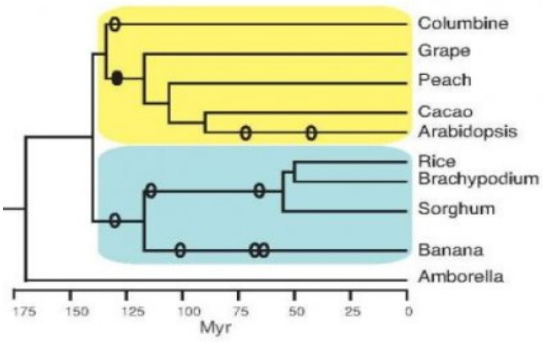
To help understand the importance of this large amount of non-coding DNA in plants, Diane Burgess and Michael Freeling at the University of California, Berkeley have identified numerous conserved non-coding sequences (CNSs) of DNA that are found in a wide variety of plant species, including rice, banana, and cacao. DNA sequences that are highly conserved, meaning that they are identical or nearly so in a variety of organisms, are likely to have important functions in basic biological processes. For example, the gene encoding ribosomal RNA, an essential part of the protein-synthesizing machinery needed by cells of all organisms, is highly conserved. Changes in the sequence of this key molecule are poorly tolerated, so ribosomal RNA sequences have changed relatively little over millions of years of evolution.

To identify the most highly conserved plant CNSs, Burgess and Freeling compared the genome (one copy of all the DNA in an organism) of the model plant *Arabidopsis*, a member of the mustard family, with the genome of columbine, a distantly related plant of the buttercup family. The phylogenetic tree (see figure) shows the evolutionary relationships among the dicot (yellow) and monocot (blue) species they studied. Branch points represent points of divergence of two species from a common ancestor. Sequences in common between these two plants, which diverged over 130 million years ago, are likely to have important functions or they would have been lost due to random mutations or insertions or deletions.

They found over 200 CNSs in common between these distantly related species. In addition, 59 of these CNSs were also found in monocots, which are even more distant evolutionarily, and these were termed deep CNSs. Finally, they showed that 51 of these appear to be found in all flowering plants, based on their occurrence in Amborella, a flowering plant that diverged from all of the above plants even before the monocot-dicot split (see figure).

So what could be the function of these deep CNSs? We can get clues by analyzing the types of genes with which these CNSs are associated. The researchers found that nearly all of the deep CNSs are associated with genes involved in basic and universal biological processes in flowering plants processes such as development, response to hormones, and regulation of gene expression. They found that the majority of these CNSs are associated with genes involved in tissue and organ development, post-embryonic differentiation, flowering, and production of reproductive structures. Others are associated with hormone- and salt-responsive genes or with genes encoding transcription factors, which are regulatory proteins that control gene expression by turning other genes on and off.

In addition, they showed that these CNSs are enriched for binding sites for transcription factors, and propose that the function of some of this non-coding DNA is to act as a scaffold for organization of the gene expression machinery. The binding sites they found are known sequences implicated in other plants as necessary for response to biotic and abiotic stress, light, and hormones. Furthermore, they discovered that a number of the CNSs could produce RNAs that have extensive double-stranded regions. These double-stranded regions have been shown to be involved in RNA stability, degradation, and in regulation of gene expression. Twelve of the most 59 highly conserved CNSs are associated with genes whose protein products interact with RNA. Clearly, these DNA sequences are not merely "junk!"



This image shows the evolutionary relationships among the species analyzed for conserved non-coding sequences. 'Myr' stands for million years ago. Ellipses are approximate times of whole-genome duplications.
(Image Credit: Diane Burgess**)**

Now that Burgess and Freeling have identified the most highly conserved non-coding DNA sequences in flowering plants, future scientists will have a better idea of which regions of the genome to focus on for functional studies. Do the predicted transcription factor-binding sites actually bind known or novel transcription factors? Do CNSs organize or regulate the gene expression machinery? Do CNSs encode RNAs that regulate fundamental processes in plants? The answers to these and many related questions will be easier to answer now that we have this set of deep CNSs that are likely to play important roles in basic cellular processes in plants.

Source: www.sciencedaily.com

Can antibiotics cause autoimmunity?

The code for every gene includes a message at the end of it that signals the translation machinery to stop. Some diseases, such as cystic fibrosis and Duchenne muscular dystrophy, can result from mutations that insert this stop signal into the middle of an essential gene, causing the resulting protein to be truncated. Some antibiotics cause the cell's translation machinery to ignore the stop codons and are therefore being explored as a potential therapy for these diseases. But new research reported online in *Proceedings of the National Academy of Sciences* shows that this approach could come with the price of triggering autoimmune disease.

"It's worth thinking about this as a potential mechanism for autoimmunity," says co-lead investigator, Dr. Laurence Eisenlohr, Professor in the Department of Microbiology and Immunology at Thomas Jefferson University.

Autoimmune diseases such as Crohn's disease, eczema, or lupus are caused by an immune system that attacks normal components of various tissues of the body. The immune system attacks these normal tissues just as it would attack tissue infected by a bacteria or virus. What causes the immune system to malfunction in some people but not others, however, has been a puzzle. "Often, the trigger happens years before the disease has been diagnosed," says Dr. Eisenlohr.

The researchers looked at a class of antibiotics that includes gentamicin because these antibiotics have the unique property of inducing cells to read through stop codons in the genetic code producing a longer protein product. This mechanism can help save the translation of mutated genes whose processing is interrupted by aberrant stop codons, such as in cystic fibrosis. However, when cellular machinery reads through normal stop codons, it could create abnormally elongated proteins in the cell. Pieces of these abnormal proteins may be presented to the immune system as a part of normal protein processing, where they could be detected by the immune system. At least, that's the theory.

To test this theory, Eisenlohr's team, in collaboration with a translation biology group at the University of Utah led by Dr. Michael Howard, used a gene that they knew would get presented to the immune system and added a stop codon in the middle of it. They then inserted this gene into a mammalian cell line. Because the stop codon truncates the gene, normal cells did not produce the protein. However, when the researchers treated the cells with gentamicin, they began to detect the protein on the surface of cells.

While a very low number of these proteins were produced too little to detect by normal biochemical tests, the T cells of the immune system are sensitive enough to pick up these miniscule amounts. Indeed, the group showed that the immune cells could detect the protein produced by gentamicin-treated cells, even at low quantities.

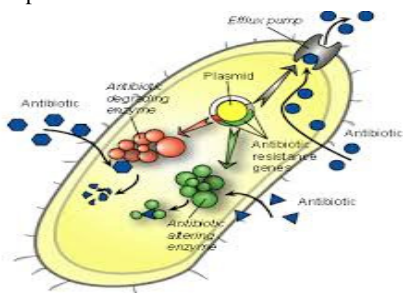
To test whether this process was active even in normal cells that weren't expressing an experimental gene, first author Elliot Goodenough exposed the HeLa human cell line to gentamicin and then searched for novel peptides presented on the surface of the cells. He identified 17 peptides that hadn't been characterized before in cells treated with gentamicin and showed that the peptides were presentable to the immune system. "The results suggest that gentamicin can cause cells to display novel protein fragments to the immune system," says Goodenough. In other words, "what may be garbage biologically may be important immunologically," says Eisenlohr.

A Few Drug-Resistant Bacteria May Keep the Whole Colony Alive

Drug-resistant mutant bacteria produce large amount of compounds called indoles that can protect large numbers of nonresistant colony mates. In a bacterial population exposed to a killer drug, a few lucky individuals might have a genetic mutation that kept them alive. They survived to reproduce, while the rest of the population perished. In short order, the entire colony consisted only of the offspring of the drug-resistant founders.

But a new study finds that just a few resistant mutants can protect large numbers of normal bacteria that would have been thought to be susceptible to the drug therapy. The research appears in the journal *Nature*.

The key seems to be indoles, which help bacteria tough out tough times. And the indoles from the mutants buck up the regular, nonresistant bacteria. The mutants themselves seem to be acting altruistically—their own growth is slowed by their indole production.



Source: www.scientificamerican.com

However, presenting an antigen to the immune system does not guarantee that it will activate the kind of immune response that initiates autoimmunity. But because gentamicin is usually used to treat infections, "all of the right conditions are in place to potentially initiate autoimmunity," says Eisenlohr. The inflammation associated with bacterial diseases gives a signal to immune cells that the peptides they encounter are dangerous. So even as gentamicin fights the bacteria causing the infection, it also causes normal cells to produce abnormal proteins that are presented to the immune system and have a potential of initiating an autoimmune reaction.

"A number of autoimmune diseases are thought to be triggered by infections," says Eisenlohr. "The results of this study suggest that certain antibiotics used to treat those infections may also contribute to that trigger." The next steps, says Eisenlohr, could be to look at population data to see whether use of gentamicin correlates with higher rates of autoimmune diseases, as well as testing whether the peptides generated during gentamicin treatment actually do cause autoimmunity in a mouse model of the disease.

Source: www.sciencedaily.com

NEWS

Fibre-rich diet prevents diabetes and obesity

It has been known for years that a fibre-rich diet protects the organism against obesity and diabetes, and French-Swedish team of researchers has succeeded in elucidating this mechanism, which involves the intestinal flora and the ability of the intestine to produce glucose between meals.

The study also clarified the role of the intestine and its associated microorganisms in maintaining glycaemia. They will give rise to new dietary recommendations to prevent diabetes and obesity.

Most sweet fruit and many vegetables such as salsify, cabbage or beans are rich in so-called fermentable fibres. Such fibers cannot be digested directly by the intestine but are instead fermented by intestinal bacteria into short-chain fatty acids such as propionate and butyrate, which can in fact be assimilated by our bodies.

The protective effect of these fibers is well known to researchers: animals fed a fiber-rich diet become less fat and are less likely to develop diabetes than animals fed a fiber-free diet. Nevertheless, the mechanism behind this effect has until now remained a mystery.

The team headed by Gilles Mithieux , CNRS researcher in the "Nutrition et Cerveau" unit (Inserm / Universite Claude Bernard Lyon 1), wondered whether this mechanism could be linked to the capacity of the intestine to produce glucose.

The researchers subjected rats and mice to diets enriched with fermentable fibers, or with propionate or butyrate and observed a strong induction of the expression of genes and enzymes responsible for the synthesis of glucose in the intestine.

They showed that the intestine of these animals used propionate as precursor to increase the production of glucose. Mice fed a fat- and sugar-rich diet, but supplemented with fibers, became less fat than control mice and were also protected against the development of diabetes thanks to significantly increased sensitivity to insulin.

The work sheds light on the role of the intestinal flora which, by fermenting dietary fiber, provides the intestine with precursors to produce glucose and also demonstrates the importance of the intestine in the regulation of glucose in the body.

Fibre-rich Fermented diet.
Source: www.deccanchronicle.com, January 17th, 2014.

Tattooing to deliver medicine

A group from France has published a paper recently in the journal *Scientific Reports* in which they have successfully used tattooing as the means to deliver a drug beneath the skin of a rat. The animal was infected by the microbe *Leishamania,* which attacks cells underneath the skin. Conventional methods such as applying ointments on the skin or swallowing a pill will not work since the area of infection is not easily reached.

Hence their idea of using a tattoo pin containing the antimicrobial drug. Reading about this method brought back the famous French saying "*Plus ça change, plus c'est la même chose*" meaning "the more it changes, the more it remains the same"

Tattoo, or *tatau* as the original Polynesian word has it, has been a time-honoured practice among many civilizations for millennia. Wikipedia tells us that a naturalist accompanying Captain Cook in his voyages of the 1770s described this practice in his records and mis-spelt it as tattoo, more suited to the Western way of pronunciation. The Chinese have been doing it, and so have the Egyptians and of course we in India. (As a child in Sholavandan, I recall watching specialists coming over to tattoo and brand cows and bulls). We not only use it to brand cattle but also men and women. A quick glance through Wikipedia also tells us about the detailed work of the Indian anthropologist, Dr. S.K. Baruah, who has described the sociological aspects of tattooing in Northeast India, particularly among the Apatani tribes of Arunachal Pradesh.

Tattooing has been done for a variety of purposes - as rites of passage, to mark the coming of age of a youngster into adulthood, and also both as a cosmetic (to enhance beauty) and as a disfigurement to mar the beauty of a girl. Polynesians distinguished tribes and sub-tribes are using tattoo marks. Indeed the Nazis in Hitler's Germany too tattooed Jews for identification, and also to distinguish people with various blood groups. Analysis of the mummies in Luxor in Egypt reveals extensive tattoo marks in the bodies of their kings and queens, thus establishing it to be a long practiced art.

This millennia-old practice of tattooing has come back to today's "hep crowd" as a mark of fashion. One sees movie actors and sportspersons flash not only their muscles and "carbs" but their tattoos as well. Hi-tech provides not only the pins to pierce the skin but even laser knives (Einstein, the originator of the idea of lasers, would have been intrigued, were he alive today).

Tattoo is by and large used for three major purposes: cosmetics, as identification marks and for medicinal purposes. The French work mentioned above is not that original in idea, but the latest example which succeeded in curing the rat of microbial infection. The drug used, called oleylphosphocholine, does not pass through the skin and go to the cutaneous region where the microbe has infected, hence the use of a tattoo pin containing the drug. In an earlier example nine years ago, a group from Holland was able to deliver DNA molecules beneath the skin, using tattoo methods, in order to vaccinate an animal.

Both these examples appear to follow the tradition of tattooing as medical practice. Recall the excitement about 23 years ago when "Otzi the ice man" was found in the Italian Alps? Detailed examination of his clothing, tools and arms he carried, and his body has provided considerable information about how this man of prehistoric times (3000-3500 BC) lost his way crossing the Alps and lost his life in the bitter cold. The *National Geographic* magazine had an extensive coverage of Otzi in its November 2011 issue. What is interesting in the present context is the finding of as many 57 tattoo lines on his body. Analysis of the places and pattern of these tattoo marks has suggested them to cover areas in his body most likely affected by arthritis. It would thus appear that Otzi was treated using the tattoo method, though it is not clear whether any drugs or similar substances were delivered through the tattoo needle.

The Chinese have of course practiced the method of acupuncture as a medical treatment practice since ancient times. They seem to have identified various specific locations in the body which respond in chosen manner to the puncturing needle. There is some growing evidence that some of these spots, when so excited, release neurochemicals that affect the body chemistry. Ancient India too appears to have attempted a similar practice. The *Charaka Samhita* apparently mentions a procedure termed needling and burning; whether this was a tattoo method of medical treatment is not clear. The current French and Dutch work appear to involve using not solid needles but hollow ones to contain the drug for delivery in an efficient manner; the fact that it works makes this little innovation not a hollow claim!

Source: www.thehindu.com, March 19th, 2014.

Drug resistance mechanism could impact development of two antibiotic drug candidates

The use of antibiotics is often considered among the most important advances in the treatment of human disease. Unfortunately, though, bacteria are finding ways to make a comeback. According to the Centers for Disease Control, more than two million people come down with antibiotic-resistant infections annually, and at least 23,000 die because their treatment can't stop the infection. In addition, the pipeline for

new antibiotics has grown dangerously thin.

Now, a new study by scientists from the Florida campus of The Scripps Research Institute (TSRI) has uncovered a mechanism of drug resistance. This knowledge could have a major impact on the development of a pair of highly potent new antibiotic drug candidates.

"Now, because we know the resistance mechanism, we can design elements to minimize the emergence of resistance as these promising new drug candidates are developed," said Ben Shen, a TSRI professor who led the study, which was published in the Cell Press journal *Chemistry & Biology*.

Bacteria Versus Bacteria

The study centers around a kind of bacteria known as *Streptomyces platensis*, which protects itself from other bacteria by secreting anti-bacterial substances. Interestingly, *Streptomyces platensis* belongs to a large family of antibiotic-producing bacteria that accounts for more than two-thirds of naturally occurring clinically useful antibiotics.

The antibiotic compounds secreted by *Streptomyces platensis*, which are called platensimycin and platencin and were discovered only recently, work by interfering with fatty acid synthesis. Fatty acid synthesis is essential for the production of bacterial cell walls and, consequently, the bacteria's existence. Platencin, although structurally similar to platensimycin, inhibits two separate enzymes in fatty acid synthesis instead of one.

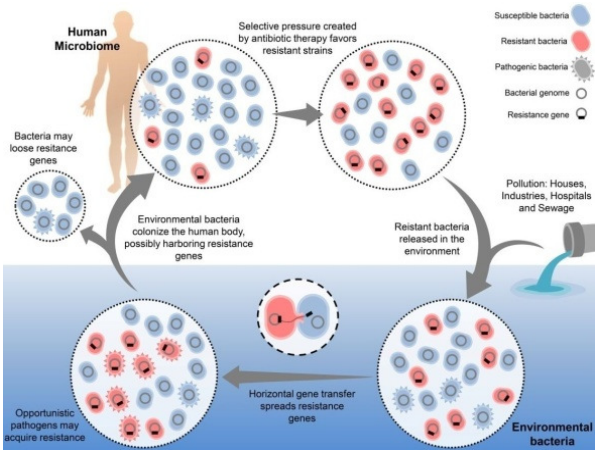
The question remained, though, of why these compounds killed other bacteria, but not the producing bacteria *Streptomyces platensis*.

The Path to Resistance

The scientists set out to solve the mystery.

"Knowing how these bacteria protect themselves, what the mechanisms of self-resistance of the bacteria are, is important because they could transfer that resistance to other bacteria," said Tingting Huang, a research associate in the Shen laboratory who was first author of the study with Ryan M. Peterson of the University of Wisconsin, Madison.

Using genetic and bioinformatic techniques, the team identified two complementary mechanisms in the bacteria that confer resistance to platensimycin and platencin. In



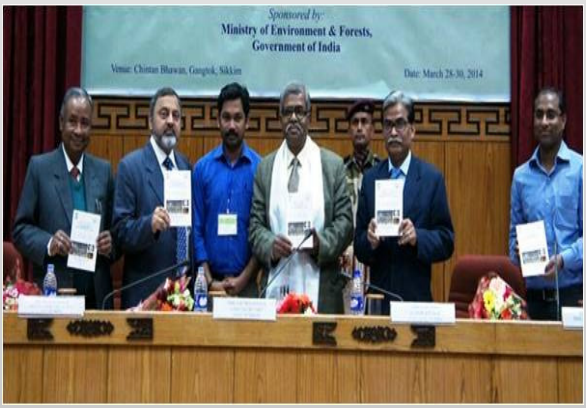
Schematic representation of the interactions between pollution resistant bacteria and aquatic environments.

essence, the study found a pair of genes in *Streptomyces platensis* exploits a pathway to radically simplify fatty acid biosynthesis while bestowing an insensitivity to these particular antibiotics.

"Understanding how these elements work is a big leap forward," added Jeffrey D. Rudolf, a research associate in the Shen lab who worked on the study. "Now these bacteria have shown us how other bacteria might use this resistance mechanism to bypass fatty acid biosynthesis inhibition."

(Source: www.sciencedaily.com**,** February 20, 2014**)**

Release of State of the Art (Series - 8) Publication of Our ENVIS Centre by the Hon'ble Chief Guest, Shri. A. K. Srivastava, Chief Secretary, Government of Sikkim



National Interaction cum Evaluation Workshop for ENVIS held at Gangtok, Sikkim | 28-30 March, 2014.

Abstracts of Recent Publications

01. Advances in Experimental Medicine and Biology, 2014, **807**, Pages: 97- 110.

Bacteriophages as Potential Treatment Option for Antibiotic Resistant Bacteria. Robert Bragg, Wouter van der Westhuizen, Ji-Yun Lee, Elke Coetsee, Charlotte Boucher

Central Department of Chemistry, Tribhuvan University.

The world is facing an ever-increasing problem with antibiotic resistant bacteria and we are rapidly heading for a post-antibiotic era. There is an urgent need to investigate alterative treatment options while there are still a few antibiotics left. Bacteriophages are viruses that specifically target bacteria. Before the development of antibiotics, some efforts were made to use bacteriophages as a treatment option, but most of this research stopped soon after the discovery of antibiotics. There are two different replication options which bacteriophages employ. These are the lytic and lysogenic life cycles. Both these life cycles have potential as treatment options. There are various advantages and disadvantages to the use of bacteriophages as treatment options. The main advantage is the specificity of bacteriophages and treatments can be designed to specifically target pathogenic bacteria while not negatively affecting the normal microbiota. There are various advantages to this. However, the high level of specificity also creates potential problems, the main being the requirement of highly specific diagnostic procedures. Another potential problem with phage therapy includes the development of immunity and limitations with the registration of phage therapy options. The latter is driving research toward the expression of phage genes which break the bacterial cell wall, which could then be used as a treatment option. Various aspects of phage therapy have been investigated in studies undertaken by our research group. We have investigated specificity of phages to various avian pathogenic *E. coli* isolates.

Furthermore, the exciting NanoSAM technology has beenemployed to investigate bacteriophage replication and aspects of this will be discussed.

Keywords: Bacteriophage; Therapy; Antibiotic resistance; *Escherichia coli*; NanoSAM

02. Diagnostic Microbiology and Infectious Disease, 2014, **79**(1) Page: 73 - 76.

Prior colonization is associated with increased risk of antibiotic-resistant Gram-negative bacteremia in cancer patients. Aaron S. Hess, Michael Kleinberg, John D. Sorkin, Giora Netzer, Jennifer K. Johnson,Michelle Shardell, Kerri A. Thom, Anthony D. Harris, Mary-Claire Roghmann

University of Maryland School of Medicine, 685 W. Baltimore Street, Baltimore, MD, 21201.

We hypothesized that prior colonization with antibiotic-resistant Gram-negative bacteria is associated with increased risk of subsequent antibiotic-resistant Gram-negative bacteremia among cancer patients. We performed a matched case-control study. Cases were cancer patients with a blood culture positive for antibiotic-resistant Gram-negative bacteria. Controls were cancer patients with a blood culture not positive for antibiotic-resistant Gram-negative bacteria. Prior colonization was defined as any antibiotic-resistant Gram-negative bacteria in surveillance or non-sterile-site cultures obtained 2–365 days before the bacteremia. Thirty-two (37%) of 86 cases and 27 (8%) of 323 matched controls were previously colonized by any antibiotic-resistant Gram-negative bacteria. Prior colonization was strongly associated with antibiotic-resistant Gram-negative bacteremia (odds ratio [OR] 7.2, 95% confidence interval [CI] 3.5–14.7) after controlling for recent treatment with piperacillin-tazobactam (OR 2.5, 95% CI 1.3–4.8). In these patients with suspected bacteremia, prior cultures may predict increased risk of antibiotic-resistant Gram-negative bacteremia.

Keywords: Antimicrobial resistance; Surveillance cultures; Neutropenic fever

E - Resources on Microorganisms

NATIONAL

Learn the issues of radionuclecides
http://www2.epa.gov/learn-issues

Biofouling solutions
http://www.biofoulingsolutions.com.au/

Antibiotic resistant organisms in health care settings
http://www.dhs.wisconsin.gov/publications/P4/P42513.pdf

Environmental, Health & Safety Laws and Regulations
http://www.michigan.gov/deq/0,1607,7-135-3310_4148-15820--,00.html

INTERNATIONAL

Microbiological Activity in Native Soils
http://www.pmac.net/stoneage.htm

The Bacteriophage Ecology Group
http://www.phage.org/

Society for Industrial Microbiology
http://www.simhq.org/

The Microbiology Information Portal
http://www.microbes.info/

EVENTS
Conferences / Seminars / Meetings 2014

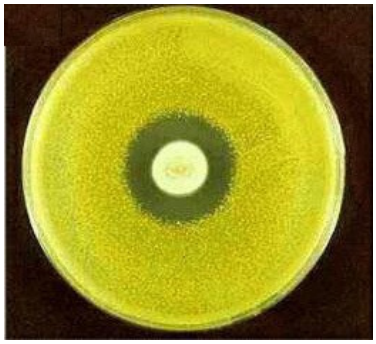
Molecular Biology of Archaea. May 19 - 22, 2014. **Venue:** Paris, **France. Website:** http://www.archaea4.org/

Retroviruses. May 19 - 24, 2014. **Venue:** Cold Spring Harbor, NY, **USA.**
Website: http://meetings.cshl.edu/meetings/2014/retro14.shtml

11th IWA Leading Edge Conference on Water and Wastewater Technologies, LET 2014. May 26 - 30, 2014. **Venue:** Abu Dhabi, **UAE. Website:** http://www.iwahq.org/26d/events/iwa-events/2014/let2014.html

Genomic Epidemiology of Malaria. June 08 - 11, 2014. **Venue:** Cambridge, **UK.**
Website: https://registration.hinxton.wellcome.ac.uk/display_info.asp?id=387

2nd International Conference on Biogas Microbiology ICBM. June 10 - 12, 2014. **Venue:** Uppsala, **Sweden.**
Website: http://www-conference.slu.se/biogas2014/



In the center of the plate is a colony of *Penicillium notatum*, a mold that produces penicillin. After appearance of the mold colony, the plate was overlaid with a bacterial culture of *Micrococcus luteus* which forms a yellow "lawn" of growth. A zone of inhibition of bacterial growth surrounds the fungal colony where penicillin has diffused into the medium.

Source: www.textbookofbacteriology.net

Bacterial Resistance to Antibiotics
History of antibiotics and emergence of antibiotic resistance

The first antibiotic, penicillin, was discovered in 1929 by Sir Alexander Fleming, who observed inhibition of staphylococci on an agar plate contaminated by a *Penicillium* mold. and he named the active substance penicillin but was unable to isolate it.

Several years later, in 1939, Ernst Chain and Howard Florey isolated penicillin and used it to treat bacterial infections during the Second World War. The new drug came into clinical usage in 1946 and made a huge impact on public health. For these discoveries Fleming, Chain and Florey were awarded the Nobel prize in 1945. Their discovery and development revolutionized modern medicine and paved the way for the development of many more natural antibiotics.

While Fleming was working on penicillin, Gerhard Domagk, a German doctor, discovered a synthetic molecule with antibacterial properties, called Prontosil, and it became the first of a long series of synthetic antibiotics called sulfonamides or sulfa drugs. Prontosil was introduced to clinical use in the 1930s and was used to combat urinary tract infections, pneumonia and other conditions. While sulfa drugs in many cases are not as effective as natural antibiotics, they are now in widespread use for the treatment of many conditions. Gerhard Domagk was awarded the Nobel prize in 1939 for his discovery of Prontosil.

Initially, the antibiotic was effective against all sorts of infections caused by *staphylococci* and *streptococci*. Penicillin had unbelievable ability to kill these bacterial pathogens without harming the host.

In the late 1940s and early 1950s, new antibiotics were introduced, including streptomycin, chloramphenicol and tetracycline, and the age of antibiotic chemotherapy came into full being. These antibiotics were effective against the full array of bacterial pathogens including Gram-positive and Gram-negative bacteria, intracellular parasites, and the tuberculosis bacillus. Synthetic antimicrobial agents such as the "sulfa drugs" (sulfonamides) and anti-tuberculosis drugs, such as para aminosalicylic acid (PAS) and isoniazid (INH), were also brought into wider usage.

DENGUE FEVER

(Aedes aegypti)